

Recurrence Phenomena After Immunoglobulin Therapy for Snake Envenomations: Part 1. Pharmacokinetics and Pharmacodynamics of Immunoglobulin Antivenoms and Related Antibodies

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The production of immunoglobulin antivenoms has evolved over the past 50 years, resulting in a choice of source animals and highly purified, target-specific immunoglobulin fragments (IgG, Fab₂, and Fab). Differences in pharmacokinetic and pharmacodynamic properties of these fragments may affect clinical efficacy. For example, both local and systemic recurrences (worsening after initial improvement) with intact or fragmented immunoglobulin antivenoms have been observed. Local recurrence may result in greater tissue injury, and coagulopathic recurrence may result in the risk of hemorrhage. The latter is of particular concern because coagulopathic recurrence usually occurs after patient discharge. Similar phenomena of symptom recurrence have been observed with ovine, digoxin-specific Fab, and with Fab₂ and IgG antivenoms from a variety of source animals as well. Recurrence of venom effects in Fab-treated patients appears to be the result of a pharmacokinetic and pharmacodynamic mismatch between the antivenom and target venom components. That is, tissue penetration and venom neutralization is incomplete, and clearance of unbound antivenom (antivenom that has not bound its venom target) is significantly faster than the clearance of some venom components, allowing signs and symptoms of envenomation to recur. Understanding the relative kinetics and dynamics of immunoglobulins and their targets may allow the physician to anticipate their clinical implications and may suggest modifications of the drug or dose to produce better clinical results.

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INTRODUCTION

Immunoglobulin antivenoms have been in clinical use for many decades. The pharmacokinetics (changes of drug concentration in the body with respect to absorption, distribution, metabolism, and excretion) and pharmacodynamics (the interaction of the drug with biologic receptors and mechanisms of action as the basis for therapeutic or toxic effects) of these agents in relation to the kinetics and dynamics of their target substances determine their clinical efficacy.

The original immunoglobulin products were minimally purified animal sera containing whole IgG, usually of equine (horse) origin. More recently, specific antibody fragments of IgG—Fab and Fab₂—produced by enzyme digestion of IgG, have been produced for a variety of venoms, toxins, and toxicants, and from a variety of animals. Fab and Fab₂ fragments are of smaller molecular weight and are not as immunogenic, with the potential advantages of decreased immediate and delayed hypersensitivity reactions and increased interstitial tissue penetration.^{1,2}

Local and coagulopathic recurrence (worsening after initial improvement following immunoglobulin therapy) has been observed during clinical trials with an affinity-purified, mixed monospecific crotaline antivenom, ovine (sheep) Fab for injection (FabAV [CroFab, Protherics]).³⁻⁶ Similar phenomena have been observed with other Fab antivenoms, digoxin-specific Fab, and with Fab₂ and IgG antivenoms as well.⁷⁻⁹ The cause of this phenomenon appears to be differences between the kinetics and dynamics of the immunoglobulin and its target.^{3,10} In the case of FabAV, the clearance of unbound antivenom is significantly faster than the absorption and clearance of some venom components, allowing signs and symptoms to recur. An understanding of the kinetics and dynamics of immunoglobulins and their targets allows assessment of their clinical implications and may suggest modifications of immunoglobulin structure and dose regimens to optimize clinical results.

GENERAL KINETICS AND DYNAMICS OF IMMUNOGLOBULINS

Every immunoglobulin has relatively unique kinetics and dynamics based on its class (eg, IgG, Fab₂, Fab), source animal, valence (monovalent, mixed monovalent, or polyvalent), route of administration, and the specific target toxin or toxicant. Also, individuals may display variable kinetics and dynamics with any particular immuno-

globulin, depending on physiologic parameters such as renal function (Table).

Immunoglobulin kinetic data generally fit a 2-compartment model, with a biexponential curve on a log-linear plot.¹¹ In general, the larger the molecule, the smaller the volume it distributes into and the longer the distribution time and elimination half-life ($t_{1/2\beta}$).¹² Disposition of the toxin/immunoglobulin complex is dependent on the size and charge of the complex, with varying degrees of elimination by the kidney and the reticuloendothelial system (RES).^{7,13}

Volume of distribution (Vd) is most dependent on molecular weight. In general, IgG distributes to a volume close to the vascular volume (~90 mL/kg). The smaller Fab₂ and Fab fragments distribute into a volume closer to the extracellular volume (~200 to 400 mL/kg).^{8,12-14}

The initial distribution phase of immunoglobulins after intravenous injection is primarily dependent on molecular weight, with larger molecules having slower distributive times. Fab distributes more rapidly and produces a higher interstitial/plasma concentration ratio than IgG or Fab₂. IgG and Fab₂ have similar distribution times and concentration ratios.^{12,15}

Immunoglobulin elimination, particularly in relation to target (toxin) elimination, is responsible for a large part of clinical response. The $t_{1/2\beta}$, the time required to reduce the blood concentration by half during the elimination phase, is largely size-dependent, with smaller molecules having faster elimination.¹⁴⁻¹⁷ The larger molecules, IgG and Fab₂, are eliminated in large part by nonrenal processes. Fab, a protein of less than 70,000 daltons, is more likely to be eliminated renally.¹⁸ The proximal tubules are also a site of protein catabolism.^{16,19} Charge and source/recipient factors, however, also affect the routes of elimination of the various immunoglobulins.²⁰⁻²² For example, renal clearance of a digoxin-specific ovine Fab in human beings was 56%.¹⁵ In contrast, IgG had 72.8% gut and 20.5% liver catabolism in the same model.¹²

Total body clearance (CL_b, the amount of drug removed per unit time by all pathways) is also generally size-dependent but is not solely a function of $t_{1/2\beta}$. In 26 human beings envenomated by the Malayan pit viper, even though the mean $t_{1/2\beta}$ of equine Fab₂ was slightly longer than equine IgG (96 versus 82 hours, respectively) and more than twice as long as caprine (goat) IgG (45.5 hours), clearance of the equine Fab₂ was higher than the caprine IgG (1.67 versus 1.3 mL/h per kilogram), and almost 3 times the clearance of the equine IgG (0.63 mL/h per kilogram).⁸

IgG has a typically long half-life in human beings. The crotaline snake antivenom currently available in the United States, Antivenin (Crotalidae) Polyvalent (Wyeth-Ayerst), has a half-life of 61 to 194 hours in envenomated patients.^{23,24} It has been detected in the urine up to 4 months after treatment.²⁵ The half-life of ovine Fab in human beings is typically much shorter. In a case series of digoxin-toxic patients, the $t_{1/2}$ of ovine, digoxin-specific Fab was 14.3 to 25.4 hours.¹³

Immunoglobulin kinetics can be affected by interactions with their target substances. Venom components are frequently larger than 20,000 daltons and can range up to 130,000 daltons.²⁶ The Fab/venom complex is too large for renal elimination and therefore remains in circulation longer than free Fab (Fab that has not bound to its target). If there is only partial neutralization of target molecules or if immunoglobulin/target dissociation occurs, this may result in increased or recurrent toxicity.

Aside from basic kinetics, the efficacy of immunoglobulins is a function of their specificity and affinity for venom components, molecular stability, the possible presence of anti-idiotypic antibodies (antibodies produced by the recipient against the therapeutic immunoglobulin), and distribution and concentration at the sites of action. Immunoglobulins generally are of high specificity and usually have significantly higher affinity for their targets than their targets have for body tissues.⁷ In other words, immunoglobulins bind their target and usually neutralize their activity.^{7,27}

Immunoglobulin antibodies appear to maintain the ability to bind their targets as long as they are in circulation.⁷ However, Fab₂ antibodies may break apart at the hinge region, affecting kinetics and dynamics. The degree of cross-linking around the hinge region is a key component in conferring longer half-life and binding affinity and may be artificially manipulated during Fab₂ preparation.²⁸

Table.

Immunoglobulin pharmacokinetics in human beings after intravenous administration.

Immunoglobulin Type	Valence	Target	Source	V_{dss} (V_d)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	Clearance (mL/min)	Notes
IgG	Polyvalent	Crotalus/Apisrodon	Horse	158.4				
IgG	Monovalent	<i>C. rhodostoma</i>	Horse	90 mL/kg	0.46	82	0.525	Single patient with <i>C. atrox</i> envenomation ²⁴
IgG	Monovalent	<i>C. rhodostoma</i>	Goat	92.5 mL/kg	1.96	45.5	1.118	7 patients; unpurified horse serum ⁸
Fab ₂	Monovalent	<i>C. rhodostoma</i>	Horse	233 mL/kg	0.3	96	1.279	6 patients; processing to minimize aggregate formation ⁴⁵
Fab ₂	Polyvalent	<i>Echis</i>	Horse			18		5 patients; pepsin-digested serum ⁸
Fab	Mixed monovalent	Crotalus/Apisrodon	Sheep	110 mL/kg	2.7	18	5.7	17 patients with <i>Echis ocellatus</i> envenomation ¹⁰
Fab	Monovalent	<i>Echis ocellatus</i>	Sheep			4.3		4 patients with <i>Crotalus snakebites</i> ²⁴
Fab	Monovalent	Digoxin	Sheep	10.8 L		12.1	23.4	22 patients with <i>E. ocellatus</i> envenomation ¹⁰
Fab	Monovalent	Digoxin	Sheep	430 mL/kg (V_d)		14	0.324 mL/min/kg	1 patient with digoxin toxicity ⁴⁷
Fab	Monovalent	Digoxin	Sheep	369 mL/kg (V_d)		9.3	0.389 mL/min/kg	7 patients with digoxin toxicity and normal renal function (creatinine clearance=103 ¹³)
Fab	Monovalent	Digoxin	Sheep	669 mL/kg (V_d)		23.9	7.1	4 patients with digoxin toxicity and impaired renal function (creatinine clearance=38 ¹³)
Fab	Monovalent	Digoxin	Sheep	2,751 mL/kg (V_d)		56.3	6.3	Treatment of digoxin toxicity in patient with serum creatinine=1.4 ⁴⁸
Fab	Monovalent	Digoxin	Sheep	95 mL/kg (V_d)		71.8	2.6	Treatment of digoxin toxicity in patient with serum creatinine=1.8 ⁴⁸
Fab	Monovalent	Digoxin	Sheep	163 mL/kg (V_d)		45.6	2.3	Treatment of digoxin toxicity in patient receiving hemodialysis ⁴⁸
Fab	Monovalent	Digoxin	Sheep	290 mL/kg		82	0.049	4 patients with digoxin toxicity, end-stage renal disease, and hemodialysis ⁷
Fab	Monovalent	Digoxin	Sheep	36.2 L		49.9	3.02	1 patient with digoxin toxicity, end-stage renal disease (creatinine clearance 8 mL/min), and peritoneal dialysis ⁴⁹

Antivenin (Crotalidae) Polyvalent (IgG) and CroFab (Fab) shown in bold type.

V_{dss} , Steady-state volume of distribution; $t_{1/2\alpha}$, distribution half-life; $t_{1/2\beta}$, terminal elimination half-life; **polyvalent**, immunogen contains multiple chemicals (eg, venom components); **monovalent**, immunogen contains only one chemical target (eg, digoxin).

The increased penetration of Fab and Fab₂ into the interstitial space, compared with IgG, suggests that such fragments would be better able to neutralize venom, toxins, or toxicants deposited there.^{12,14} Unfortunately, this property does not appear to confer enhanced efficacy in treating local venom effects in snakebite envenomations. In vitro, IgG, Fab₁, and Fab are similarly effective in neutralizing locally active venom components. Fab and Fab₂ antibody fragments to *Vipera berus* (European viper) were equal in neutralizing the hemorrhagic tissue effects of the venom in mice.²⁹ Equine IgG and Fab₂ against *Bothrops asper* (barba amarilla) were protective in mice when mixed with venom before injection, but were only partially and equally effective in neutralizing local tissue damage when injected at different sites from the venom. Even when the antivenom was given before injecting the venom, neither agent was capable of neutralizing all of the venom at the bite site. Although venom-induced capillary disruption may result in extravasation of immunoglobulin into the injured tissue, most neutralization appears to take place at the leading edge of tissue injury and in the central circulation.³⁰

In contrast to local effects, antivenom administered 5 or 120 minutes before envenomation completely prevented coagulopathic effects,³⁰ suggesting that coagulopathic toxicity occurs, and can be prevented, in the central circulation. Thus, all intravenously administered antivenoms should potentially be capable of initial neutralization of coagulopathic venom components.

A closely analogous situation occurs in patients treated with a digoxin-specific Fab, who exhibit a similar recurrence phenomenon to that of antivenoms. Free digoxin concentrations fall rapidly after Fab administration, but then rebound upward in patients with normal renal function within 12 to 24 hours.⁷ In patients with renal failure, that rebound is delayed 12 to 130 hours.⁷ The possible reasons for the rebound include redistribution of unbound digoxin from tissue stores after unbound antibody elimination, loss of binding ability of the antibody, and dissociation of the Fab/digoxin complex. Dissociation between Fab and digoxin is an unlikely explanation because the binding affinity makes the expected rate of dissociation too small to explain the rebound. Also, the free fraction of digoxin in the presence of Fab remains less than the free fraction in the absence of Fab, indicating continuous binding as long as antibody is present. The most likely explanation in patients with normal renal function is that unbound Fab is eliminated shortly after administration. If free digoxin then redistributed into the central circulation, there would be no Fab available to

bind it and free digoxin levels would rebound. Because unbound Fab excretion is impaired in renal failure, a delay to rebound would be expected, as free Fab would still be present to bind redistributing unbound digoxin as it reentered the blood. Rebound of digoxin levels would only occur if and when the excess Fab was completely occupied.

Finally, anti-idiotypic antibodies may develop in the immunoglobulin recipient. An anti-idiotypic antibody forms against the therapeutic antibody, thereby preventing the therapeutic antibody from binding its target molecule. Development of anti-idiotypic antibodies usually requires 1 to several weeks and thus they are unlikely to play a role in the recurrence phenomenon seen in response to a first exposure to an antibody.³¹ However, they may cause decreased clinical efficacy in subsequent antibody use.¹

In preliminary work, unbound FabAV administered during a clinical trial of crotaline envenomation had a $t_{1/2\alpha}$ of 2.5 to 2.7 hours, $t_{1/2\beta}$ of 18 hours, Cl_R of 4.9 mL/hr per kilogram, and Vd of 110 mL/kg, consistent with other ovine Fab preparations (Table).³²⁻³⁴

VENOM KINETICS AND DYNAMICS

Snake venom is made up of dozens of components of varying molecular weights and activities.^{35,36} No systematic study of venom component kinetics and dynamics has been undertaken. However, in case reports and case series of snake envenomation, patients exhibit prolonged coagulopathic manifestations, with coagulopathies persisting up to 2 weeks after envenomation, suggesting that coagulopathic components of snake venom remain present and active in the body during this time.³⁷⁻⁴² A case of envenomation by *Crotalus atrox* (Western diamondback rattlesnake) was reported in which venom antigen was detectable in urine at least 4 to 6 days after presentation.²¹ Thrombocytopenia has persisted until at least day 10 in a timber rattlesnake envenomation,³⁸ for 7 to 14 days after envenomation by *Crotalus horridus horridus* (timber rattlesnake),⁴¹ for 8 days after a *Crotalus ruber ruber* (red diamond rattlesnake) bite,³⁹ and for at least 6 days after envenomations by *Crotalus viridis helleri* (southern Pacific rattlesnake).⁴² A case of a *C. atrox* envenomation treated without antivenom had persistently abnormal prothrombin time, thrombin time, and reptilase time 8 days after the bite.⁴⁰ In most of these reports, the coagulopathy was still present when coagulation monitoring was discontinued.

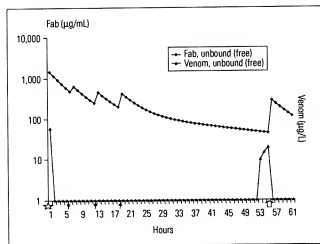
Venom distribution and activity are altered by antivenom administration. An equine Fab₂ against *Vipera* sp

administered to human beings resulted in a 2.3-fold elevation of the plasma venom concentration and a decline in the $t_{1/2\beta}$ of the venom from 31.92 hours to 16.73 hours compared with the venom alone.⁴³ At the same time, all unbound venom in the central circulation becomes bound to the antibody.^{3,8,29} Thus, antivenom depletes unbound venom in the body and increases total body clearance of venom.

Despite the early use of antivenom, it appears that a depot of unneutralized venom often remains in the body, and therefore prolonged absorption of venom components may occur. The clearance of initially unbound FabAV is much more rapid than that of unbound venom, creating the conditions for recurrent venom antigenemia and its effects (Figure 1). Likewise, recurrent venom antigenemia and its effects may be seen in patients treated with IgG,⁷ most likely from an eventual exhaustion of available unbound IgG with unneutralized venom continuing to enter the central circulation (Figure 2).

Figure 1.

Idealized graph (based on preliminary kinetic data³⁵) of plasma concentrations of unbound Fab crotaline antivenom (FabAV, diamonds), and unbound venom antigens (triangles) versus time. The administration of 12 vials of FabAV (star) at time 0 results in immediate disappearance of unbound venom from the circulation. Redosing of 2 vials of FabAV at 6, 12, and 18 hours after initial control (solid arrows) prevents local recurrence. Venom antigenemia is not required for local recurrence. When unbound antivenom concentrations fall below some critical level, venom antigenemia may recur, with recurrence of venom coagulopathic effects. Redosing of 2 vials of FabAV at this time (open arrow) may again result in immediate disappearance of unbound venom from circulation.

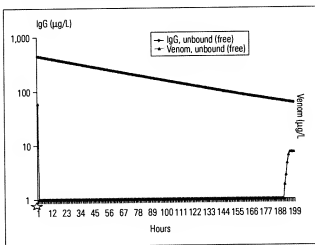


IMMUNOGLOBULIN STRUCTURAL OR DOSE REGIMEN MODIFICATIONS

Modifications in antivenom production, structure, or dose regimens might improve the venom/antivenom kinetic mismatch and prevent rebound of serum toxicant levels or recurrent venom effects. For example, a second dose of digoxin-specific Fab 7 hours after the initial dose resulted in an increase in bound Fab from 47.6 to 70.2%.¹³ In the FabAV clinical trials, administration of scheduled doses of FabAV during the first 18 hours after the initial dose prevented recurrence of local symptoms.³³ Also, it has been proposed that it is theoretically possible to maintain a steady-state blood immunoglobulin concentration that is protective against late recurrence of coagulopathy.³⁴ Intramuscular injection would likely extend the apparent $t_{1/2\beta}$. Equine Fab₂ $t_{1/2\beta}$ increased 25% after intramuscular injection. However, it also resulted in a maximum plasma concentration (C_{max}) that was only

Figure 2.

Idealized graph (based on published kinetic data^{23,24}) of plasma concentrations of unbound IgG crotaline antivenom (diamonds) and unbound venom antigens (triangles) versus time. The administration of 10 vials of IgG antivenom (star) at time 0 results in immediate disappearance of unbound venom from the circulation. When antivenom concentrations fall below some critical level, venom antigenemia may recur, with recurrence of venom coagulopathic effects. Typically this occurs at a later time than with FabAV (190 hours longer to bind venom components entering the central circulation (assuming an appropriate dose of antivenom was administered).



10% of the intravenous dose, a significantly prolonged time to maximal plasma concentration, and less than half fractional drug absorption ($f=0.42$).¹⁴ Although the slow absorption rate, incomplete absorption, and low C_{max} would make this route a poor choice for initial management, intramuscular use after initial control, perhaps in sustained-absorption vehicles, may provide persistent plasma levels of antivenom sufficient to prevent recurrent coagulopathy.³⁴

Other modifications might include improved purification, which increased specific binding activity by 51%²⁶; alteration of charge or complexation with glycosate⁴⁴ or dextran, which altered biodistribution, kinetics, and catabolism⁴⁵; and coadministration of other substances such as basic amino acids, lysine, or arginine, which have been shown to block renal accumulation of Fab.⁴⁴

In summary, the pharmacokinetics and pharmacodynamics of immunoglobulins determine their clinical efficacy. Every therapeutic immunoglobulin preparation will have relatively unique kinetics and dynamics, but recurrence phenomena seen with immunoglobulin therapy are a result of differences between the kinetics and dynamics of these agents and their target toxins or toxicants. A great disparity between venom and antivenom kinetics and dynamics occurs with Fab antivenoms. The failure to neutralize all venom initially and the more rapid clearance of unbound Fab in relation to venom components may result in recurrence of local and systemic venom effects. Modification of Fab structure or dose regimen may produce a better kinetic and dynamic match between an immunoglobulin and its target.

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